

Tipo de Comunicación:

Comunicación Oral: OC\_BM\_16

Simposio:

BIOINGENIERÍA MOLECULAR, BIOLOGÍA DE SISTEMAS E INGENIERÍA METABÓLICA. NUEVAS TECNOLOGÍAS

Título:

Engineering osmotic stress tolerance in the riboflavin producer *Ashbya gossypii*

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Palabras Clave:

*Ashbya gossypii*; glycerol; hyperosmotic stress

Comunicación:

The worldwide scenario of strong investment in biodiesel production brings new economical and environmental concerns that need to be addressed. Among them, the most prominent lies on the accumulation of large amounts of a main by-product of the process, crude glycerol. Valorization of this biodiesel waste through its microbial conversion into high-value products is one of the most attractive and sustainable applications explored. Crude glycerol contains several impurities such as methanol, soaps and salts. After pre-treatment of this biodiesel waste, some impurities can disappear or be mitigated, however salts as KCl and NaCl continue to be present, which impose osmotic pressure to microorganisms. The filamentous fungus *A. gossypii* [1], used for more than 20 years for the commercial production of riboflavin, is able to metabolize glycerol and use it as its preferred solute to overcome hyperosmotic stress conditions [2]. However, *A. gossypii* exhibits great sensitivity to hyperosmotic stress when compared with other fungal species [3]. Here, we designed a strategy to improve the osmotic stress tolerance of *A. gossypii* by overexpressing native and heterologous genes involved in the uptake of glycerol. The cells were exposed to hyperosmotic stress in complex medium with glucose as carbon source and their colony radial growth was monitored. Different stress agents, ionic (NaCl and KCl) and non-ionic (sorbitol), were used in the presence or absence of glycerol to test if the overexpressing strains could efficiently utilize exogenous glycerol towards an improvement in hyperosmotic stress tolerance. In the presence of 0.8M and 1M KCl, *A. gossypii* overexpressing strains displayed significantly improved hyperosmotic stress tolerance compared to the control strain, being able to grow in these conditions due the presence of glycerol in the medium.

These results contribute to the further development of *A. gossypii* as an environmental-friendly cell factory organism, contributing to its establishment in the biorefinery concept.

1. Aguiar et al., *BiotechnolAdv* 2015; 1774-86.

2. Förster et al., *ApplMicrobiolBiotechnol* 1998; 50:219-26.

3. Nikolaou et al., *BMCEvolBiol* 2009; 9:44.

Acknowledgments to Fundação para a Ciência e a Tecnologia, Portugal: Strategic Project CEB unit UID/BIO/04469/2013 and PhD grant PD/BD/113812/2015 to R Silva.